Journal of Nuclear Medicine, published on August 12, 2019 as doi:10.2967/jnumed.119.227652

The Genetic Duet of *BRAF* V600E and *TERT* Promoter Mutations Robustly Predicts the Loss of Radioiodine Avidity in Recurrent Papillary Thyroid Cancer

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Running Title: BRAF and TERT in Radioiodine Refractoriness

Key Words: *BRAF* V600E, *TERT* promoter mutation, radioiodine therapy, thyroid cancer, recurrence

Total Word Count: 4687

Financial support

This study was supported partly by US National Institutes of Health (NIH) Grants No. R01CA215142 and R01CA189224 (M.X.)

Disclosures: Mingzhao Xing receives royalties as co-holder of a licensed USA patent related to *BRAF* V600E mutation in thyroid cancer. Other authors have no conflict of interest to disclose.

Disclaimer: The content of this article is solely the responsibility of the authors and does not necessarily reflect the official views of the funding entity.

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ABSTRACT

BRAF V600E and *TERT* promoter mutations, particularly their genetic duet, are well known to be associated with poor clinical outcomes of PTC. Loss of radioactive iodine (RAI) avidity in recurrent PTC is a major cause of treatment failure and hence poor clinical outcomes of PTC. This study investigated the role of the mutation patterns in the loss of RAI avidity in recurrent PTC.

Methods: Retrospective study of the relationship between the loss of RAI avidity in structural recurrent PTC and the genotype patterns of *BRAF* V600E and *TERT* promoter mutations in 164 patients (104 females and 60 males) with a median age of 50 (interquartile, 35-62) years.

Results: The overall prevalence of RAI avidity loss in recurrent PTC was 62.8% (103/164). When dividing the cohort into mutation and wild-type groups, RAI avidity loss was 80.4% vs. 33.9% (P<0.001) in *BRAF* V600E versus wild-type *BRAF* patients with an adjusted odds ratio (OR) of 7.11 (95% CI, 3.24-16.27) and was 89.4% vs. 52.1% (P<0.001) in *TERT* mutation versus wild-type patients with an adjusted OR of 6.89 (95% CI, 2.28-25.66). When dividing the cohort into four genotypes, RAI avidity loss was 70.3% (45/64), 55.6% (5/9), and 97.4% (37/38) in patients with *BRAF* V600E alone, *TERT* mutation alone, and the genetic duet of coexisting *BRAF* and *TERT* mutations versus 30.2% (16/53) in patients with neither mutation (P<0.001, 0.251, and <0.001, respectively). These corresponded to ORs (95% CI) of 5.39 (2.31-13.13), 2.84 (0.53-16.32), and 81.04 (11.67-3559.83), respectively. The synergy index was 13.28 (95% CI,

1.54-114.46; P=0.019) between *BRAF* V600E and *TERT* mutation in cooperatively affecting the RAI avidity. A similar genotype-associated expression pattern was observed for thyroid iodide-handling genes.

Conclusion: *BRAF* V600E alone and particularly coexisting *BRAF* V600E and *TERT* promoter mutations are strongly associated with the loss of RAI avidity and impairment of the iodide-metabolizing machinery in recurrent PTC, showing a robust predictive value for failure of RAI treatment of PTC.

INTRODUCTION

Papillary thyroid cancer (PTC), comprising about 85-90% of all thyroid cancers, is a common endocrine malignancy (*1-3*), which is histologically classified into several variants, among which conventional PTC (CPTC) accounts for the majority (*4-6*). With the current standard treatments, PTC has generally an excellent clinical prognosis (*7*). The conventional radioactive iodine (RAI) therapy using I^{131} contributes to this excellent performance of clinical treatment of PTC by reducing disease recurrence. This takes the advantage of the unique ability of thyroid cells to take up iodide through iodide-metabolizing molecules specifically expressed in thyroid cells. These include sodium-iodide symporter (NIS), which transports iodide into the thyroid cell from the blood stream, thyroid peroxidase (TPO), which oxidizes iodide into iodine, and thyroglobulin (TG) which incorporates iodine into its tyrosine residuals to produce thyroid hormone (*4*). These genes are regulated by specific transcriptional factors, such as PAX-8 (*8,9*). Normal function of this iodide-metabolizing machinery is critical for thyroid hormone biosynthesis in normal thyroid physiology and for RAI uptake and trap in cancer cells in RAI treatment of PTC, which is up-regulated by thyroid-stimulating hormone (TSH) receptor (TSHR).

There are often patients with recurrent PTC that is surgically inoperable and has lost RAI avidity due to the silencing of thyroid iodide-metabolizing genes (*7*), for which predictive molecular markers may be helpful if identified. *BRAF* V600E is a prominent oncogene in PTC and has an established prognostic value for poor prognosis of this

cancer (*4,10*). *TERT* promoter mutation, existing in two main forms, chr5, 1,295,228 C>T (C228T) and 1,295,250 C>T (C250T), is another major oncogene in PTC that is also associated with poor clinical outcomes (*11*). The genetic duet of coexisting *BRAF* V600E and *TERT* promoter mutations is particularly robustly associated with poor clinical outcomes of PTC (*12,13*). In the present study, we investigated the role and predictive value of *BRAF* V600E and *TERT* promoter mutations in primary PTC tumor for the loss of RAI avidity in subsequent recurrent disease.

MATERIALS AND METHODS

Study subjects

We previously established a large cohort of 1,051 patients with PTC to assess the relationship between genetic variants and the clinical prognosis of PTC (*13*). In this cohort, structural recurrence of PTC, defined as recurrent tumor confirmed radiographically/cytologically/pathologically, occurred in 167 patients. Among these 167 patients, 164 cases, including 104 females and 60 males with a median age of 50 (interquartile-IQR, 35-62) years, had clinical radiology reports available on the whole-body RAI imaging scan to evaluate recurrent diseases of PTC. The present study was focused on these 164 patients. The loss of RAI avidity was defined as negative RAI uptake in one or more lesions of recurrent PTC on whole body RAI scans, which included either diagnostic I^{123} body scans or I^{131} post-treatment scans. Patients were prepared for RAI body scan with either thyroid hormone withdrawal or recombinant

human TSH stimulation with a TSH level achieved >30 mIU/L. All patients received total or near-total thyroidectomy as the initial treatment. Therapeutic neck lymph node dissection and I^{131} ablation therapy after total thyroidectomy for the treatment of the initial disease were pursued as clinically indicated following standard treatment criteria as previously described (*12,13*). The study was approved by our institutional review board and informed patient consent was obtained where required.

Mutation analysis

Genomic DNA was isolated from primary PTC samples using the standard phenol-chloroform extraction and ethanol precipitation procedures. The regions harboring the spots for *BRAF* V600E and *TERT* promoter mutations in the *BRAF* and *TERT* genes were amplified by respective polymerase chain reactions, followed by Sanger sequencing to detect the *BRAF* and *TERT* promoter mutation status as described previously (*12,13*).

Analysis of the relationship between mutations and the expression of thyroid iodide-handling genes

The *BRAF* V600E and *TERT* promoter mutation status and the normalized RNA-Seq data were acquired from The Cancer Genome Atlas (TCGA) data portal (*14*). The mRNA expression level of five thyroid iodide-handing genes, including *NIS*, *TSHR*, *TPO*, *TG*, and *PAX8*, was calculated by the log-transformation of the RNA counts.

Statistical analysis

Continuous data were summarized as medians and IQRs and compared using the Wilcoxon-Mann-Whitney test. Categorical variables were summarized as frequencies and percentages and compared using the Chi-squared test. Logistic regression models were used to assess the effects of mutations on the risk of RAI avidity loss, which were adjusted for patient age and sex. The interactions between *BRAF* and *TERT* mutations in affecting the risk of RAI avidity loss were tested using the synergy index with 95% confidence intervals (CI) (*15*). Statistical analyses were performed using Stata/SE version 12 (Stata Corp). All P-values were 2-sided and P-values <0.05 were considered statistically significant.

RESULTS

Relationship between *BRAF* V600E, *TERT* promoter mutation and the loss of radioactive iodine avidity in recurrent papillary thyroid cancer

The overall prevalence of loss of RAI avidity in recurrent PTC was 62.8% (103/164). When dividing the entire cohort of the 164 patients into *BRAF* V600E-positive and -negative groups (**Table 1**), the loss of RAI avidity more commonly occurred in *BRAF* V600E-positive patients than in *BRAF* V600E-negative patients (80.4% vs. 33.9%,<0.001), with an adjusted odds ratio (OR) of 7.11 (95% CI, 3.24-16.27) for *BRAF* V600E-associated risk for the loss of RAI avidity. When dividing the entire cohort of the 164 patients into *TERT* mutation-positive and -negative groups, the

loss of RAI avidity more commonly occurred in *TERT* mutation -positive patients than in mutation-negative patients (89.4% vs. 52.1%; <0.001), with an adjusted OR of 6.89 (95% CI, 2.28-25.66) for *TERT* mutation-associated risk for the loss of RAI avidity. Similar observations were made when only CPTC was analyzed (**Table 1**).

Effects of *BRAF* V600E alone, *TERT* promoter mutation alone or the genetic duet of their coexistence on the loss of radioactive iodine avidity in recurrent papillary thyroid cancer

When dividing the 164 patients into 4 groups (**Table 2**), loss of RAI avidity was found in 70.3% (45/64), 55.6% (5/9), and 97.4% (37/38) patients with *BRAF* mutation alone, *TERT* mutation alone, and the genetic duet of coexisting *BRAF* and *TERT* mutations versus 30.2% (16/53) patients with neither mutation (P<0.001, 0.251, and <0.001, respectively). These corresponded to ORs (95% CI) of 5.39 (2.31-13.13), 2.84 (0.53-16.32), and 81.04 (11.67-3559.83), respectively, which remained similar after adjustment for patient age and sex (**Table 2**). These analyses showed that *BRAF* V600E alone had a significant effect on the loss of RAI avidity while *TERT* promoter mutation alone had no significant effect and the genetic duet of the coexisting mutations had a robust effect. Similar observations were made when only CPTC was analyzed (**Table 2**). When only *TERT* C228T (excluding *TERT* C250T) in relation to *BRAF* V600E was analyzed, similar genetic effects on RAI avidity loss in recurrent PTC were found (**Table 3**).

The risk of RAI avidity loss associated with coexisting *BRAF* and *TERT* mutations was dramatically higher than the sum of the effects of the two mutations individually, suggesting a synergistic interaction between the two mutations. Indeed, synergism analysis revealed a robust synergy index of 13.28 (95% CI, 1.54-114.46; P=0.019) between *BRAF* V600E and *TERT* promoter mutations (C228T + C250T) and a similarly robust synergy index of 10.99 (95% CI, 1.28-94.06; P=0.029) between *BRAF* V600E and *TERT* C228T (**Table 4**).

Relationship between the expression level of iodide-handling thyroid genes and the genotypes of *BRAF* V600E and *TERT* promoter mutations in papillary thyroid cancer

To explore a molecular background for the loss of RAI avidity associated with *BRAF* V600E and *TERT* mutations in PTC, we used the PTC data in the TCGA database to analyze the relationship between the genotypes of *BRAF* V600E and *TERT* promoter mutations and the expression of classical iodide-handling genes, including *NIS*, *TSHR*, *TPO*, *TG*, and *PAX8* in 386 PTC samples that had information available for the analysis. When the entire cohort was divided into two groups of *BRAF* V600E-positive and –negative cases, the expression of these thyroid iodide-metabolizing genes was all significantly lower in the mutation-positive group than the mutation-negative group (**Fig. 1A**). When the entire cohort was similarly divided into two groups of *TERT* mutation-positive and –negative cases, the expression of the thyroid iodide-metabolizing genes was

similarly all lower in the mutation-positive group than the mutation-negative group (**Fig. 1B**).

We also examined the relationship between thyroid gene expression and each mutation alone or the genetic duet of coexisting mutations by dividing the cohort into four genotype groups. As exemplified by the thyroid *TG* gene (**Fig. 2A**) and *TSHR* gene (**Fig. 2B**), compared with the group harboring neither mutation, *BRAF* V600E alone, but not *TERT* mutation alone, was associated with a significantly lower gene expression level. The gene expression level in the group with the genetic duet of *BRAF* V600E and *TERT* mutation was even lower than that in the group harboring neither mutation and, in fact, was the lowest among the four genotype groups; it was also significantly lower than the gene expression level in the groups harboring either mutation alone. Similar trends of the relationship between mutations and gene expression were found with *NIS*, *TPO*, and *PAX8* (**supplemental Fig. 1**). These patterns of the effects of the two gene mutations on thyroid gene expression mirrored those of the effects of the mutations on RAI avidity loss in recurrent PTC.

DISCUSSION

In recent years, *BRAF* V600E has been introduced as a prognostic marker for poor prognosis of PTC, including the risk for loss of RAI avidity of recurrent disease (*4,16*). In 2005, *BRAF* V600E was for the first time shown to be associated with the loss of RAI avidity in recurrent PTC (*10*), a phenomena that has now been widely observed (*4*). *In*

vitro studies functionally showed a direct link of *BRAF* V600E to the impairment or even complete silencing of thyroid iodide-metabolizing genes (*17*), which was subsequently reproduced in *in vivo* studies (*18*). *BRAF* V600E was also shown to be associated with mis-localization of NIS in the cytoplasm of thyroid cancer cells (*19*). These provide a molecular explanation for the association of *BRAF* V600E with the loss of RAI avidity in PTC and support *BRAF* V600E as an increased risk for the loss of RAI avidity in PTC.

TERT promoter mutation has now become recognized as another important oncogene and genetic prognostic marker in thyroid cancer (*11*). In particular, the initial finding of the coexistence of *TERT* promoter mutations and *BRAF* V600E in PTC in 2013 (*20*), which has been confirmed in many studies (*11*), has stimulated tremendous interest in its potential clinical and biological relevance. Indeed, the genetic duet of *BRAF* V600E and *TERT* promoter mutations has been demonstrated to have a robust synergistic adverse effect on clinical outcomes of PTC, including disease recurrence (*12*) and patient mortality (*13*). This robust synergistic oncogenic-promoting role of the two mutations uses a novel molecular mechanism that requires both *BRAF* V600E and *TERT* promoter mutations to synergistically promote the *TERT* expression by activating a novel BRAF/MAP kinase/FOS/GABP/TERT pathway system (*21*). It is thus well-established today that *BRAF* V600E and *TERT* promoter mutations are the most prominent oncogenic genetic driver events in PTC.

Different from the previous studies discussed above, the present study emphasized the investigation of the role of both *BRAF* V600E and *TERT* promoter mutations,

particularly the genetic duet of their coexistence, in RAI avidity status in recurrent PTC. We found that *BRAF* V600E alone but not *TERT* mutation alone was significantly associated with the loss of RAI avidity in recurrent PTC and the genetic duet of the two coexisting mutations had the most robust effect on the loss of RAI avidity in recurrent PTC. We also for the first time investigated the role of the four genotypes in the silencing of thyroid genes as a molecular mechanism for the loss of RAI avidity. We similarly demonstrated the most robust effect of the genetic duet of *BRAF* and *TERT* mutations on the impairment of the expression of thyroid iodide-handling genes. Interestingly, unlike *BRAF* V600E alone, *TERT* promoter mutation alone also had no significant effect on thyroid gene expression, mirroring its lack of effect on RAI avidity status. A recent elegant study demonstrated an association of *BRAF* V600E and *TERT* promoter mutations with RAI refractoriness in distant metastatic PTC (*22*). That study also showed that the genetic duet of *BRAF* V600E and *TERT* promoter mutations had the most robust effect.

Our findings of the effects of mutations on RAI avidity and thyroid gene expression in the present study are remarkably consistent with previous findings on the patterns of the effects of these mutations on clinicopathological outcomes of PTC—*BRAF* V600E alone has a significant effect while *TERT* nutation alone barely had any effect and the genetic duet of the two mutations had a robustly synergistic effect on aggressive pathological behaviors and disease recurrence of PTC (*12*). These previous clinicopathological findings with respect to the genotypes can now be explained by our present findings of the similar effects of the mutations on thyroid gene silencing and RAI

avidity loss, which makes the tumor resistant to RAI treatment, thus resulting in poor clinical outcomes (e.g., increased disease recurrence).

A limitation of this study was our inability to use the Schlumberger et al criteria to define "RAI refractoriness" (*23*) due to the incomplete information from the old clinical records of our study subjects. For example, our patients were mostly treated with only one dose of I^{131} at the first occurrence of recurrent disease, where the RAI body scans were obtained and used for the present study. Thus, the criteria of "accumulated dose of 600 mCi" in the Schlumberger et al definition could not be used. We therefore defined "loss of RAI avidity" in the present study as negative uptake of RAI in one or more recurrent lesions of PTC on RAI body scans. Also, diagnostic I^{123} body scan or I^{131} post-treatment body scan was either alone used to detect RAI avidity in our analyses, with the former being known to be associated with suboptimal sensitivities. These methodological limitations may explain the relatively high rate of loss of RAI avidity reported in the present study. Nevertheless, as a novel and large study that for the first time addresses the role and potential clinical utility of these unique genetic patterns in the context of the challenges associated with RAI treatment of recurrent thyroid cancer have important clinical ramifications. Better-controlled future studies are required to definitively establish the clinical utility of *BRAF* V600E and *TERT* promoter mutations in predicting the failure of RAI treatment of thyroid cancer.

CONCLUSION

We demonstrate here that *BRAF* V600E and *TERT* promoter mutations are synergistically associated with the loss of RAI avidity in recurrent PTC, mirroring a

similar pattern of expression impairment of thyroid iodide-handling genes and corresponding to the previously reported similar pattern of clinical outcomes associated with these genotypes. These results provide an explanation for the association between *BRAF* V600E and *TERT* promoter mutations and RAI treatment failure and poor clinical outcomes of PTC. The unique genetic duet of *BRAF* V600E and *TERT* promoter mutations represents a genetic background that may help identify patients with PTC with a potential for failure of RAI treatment of recurrent or persistent diseases. This emphasizes the importance of disease eradication in the initial treatment of PTC harboring the genetic duet to minimize the risk of persistence of RAI-refractory disease or development of RAI-refractory recurrent disease.

DISCLOSURE: Mingzhao Xing receives royalties as co-holder of a licensed USA patent related to *BRAF* V600E mutation in thyroid cancer. Other authors have no conflict of interest to disclose.

Acknowledgements

This study was supported partly by US National Institutes of Health (NIH) Grants No. R01CA215142 and R01CA189224 (M.X.). The results published here are in part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/.

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KEY POINTS

QUESTION What is the role of *BRAF* V600E and *TERT* promoter mutations, particularly their coexisting duet, in the loss of radioactive iodine (RAI) avidity in recurrent papillary thyroid cancer (PTC)?

PERTINENT FINDINGS *BRAF* V600E alone, but not *TERT* promoter mutation alone, was significantly and the genetic duet of coexisting two mutations were particularly robustly associated with the loss of RAI avidity in recurrent PTC as well as impaired expression of thyroid iodide-metabolizing genes in primary PTC.

IMPLICATIONS FOR PATIENT CARE Knowledge of the status of *BRAF* V600E and the genetic duet of coexisting *BRAF* and *TERT* mutations may help predict RAI avidity status of recurrent PTC and correspondingly help guide appropriate treatments.

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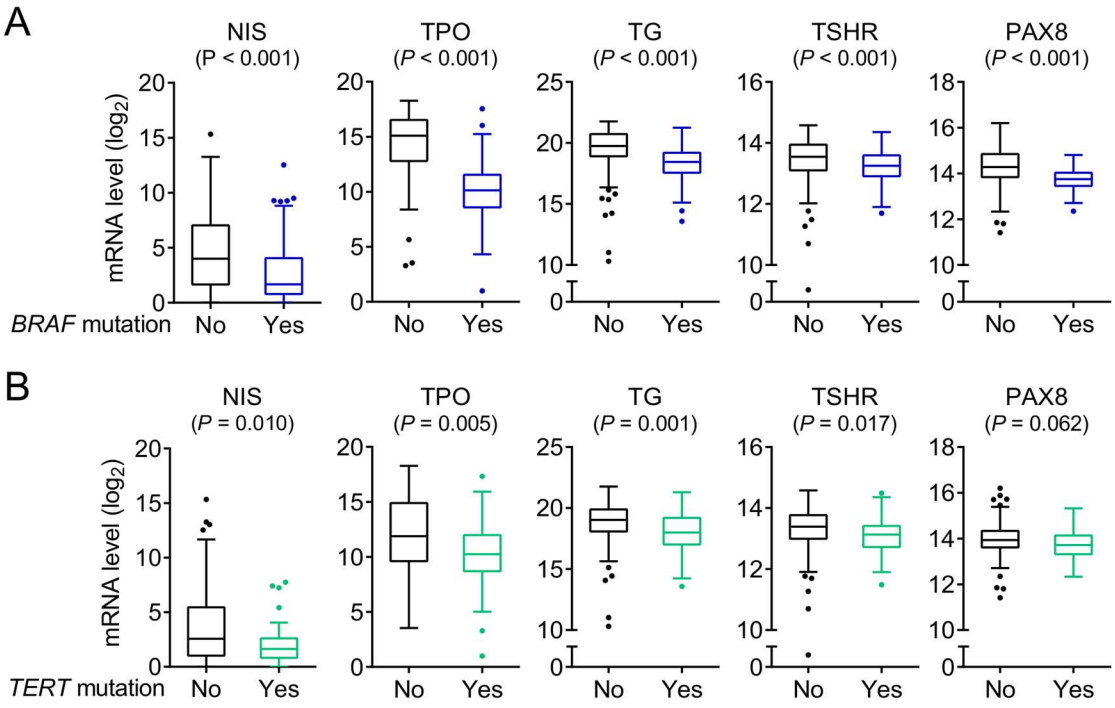


FIGURE 1. ***Box plots of mRNA expression of thyroid iodide-handing genes in papillary thyroid cancer***. **A**. Comparison of the gene expression levels between *BRAF* V600E-negative and –positive groups by dividing the entire cohort into the indicated two genotype groups. **B**. Comparison of the gene expression levels between *TERT* promoter mutation-negative and –positive groups by dividing the entire cohort into the indicated two genotype groups. The central horizontal lines represent medians and the box boundaries represent interquartile ranges. The sample sizes in *BRAF* mutation -negative and -positive groups were 160 and 226, respectively. The sample sizes in *TERT* mutation -negative and -positive groups were 347 and 39, respectively. *TERT* promoter mutations here included collectively *TERT* C228T and *TERT* C250T. *P* values were calculated using the two-sided Wilcoxon-Mann-Whitney test.

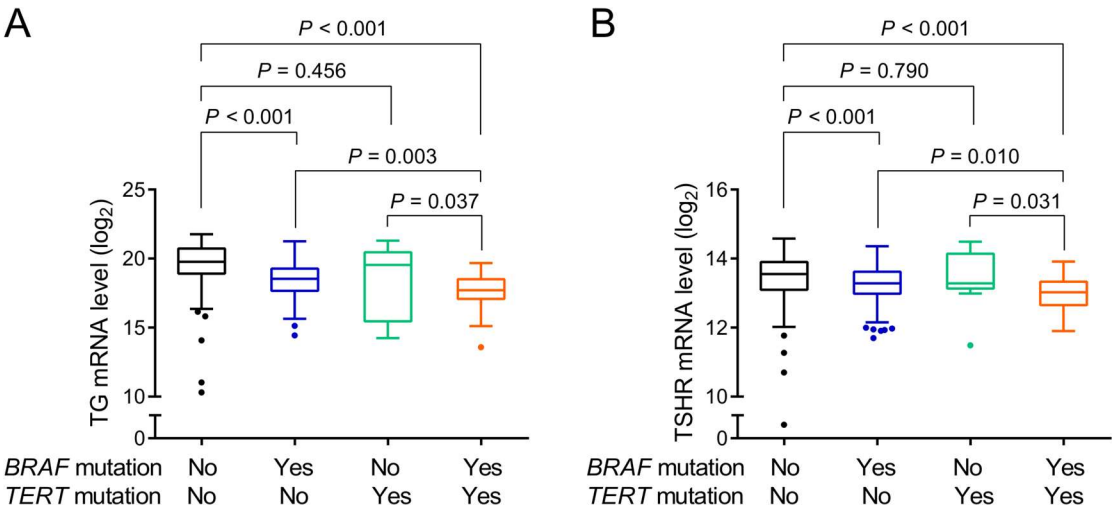


FIGURE 2. ***Box plots of mRNA expression of thyroid iodide-handling genes in papillary thyroid cancer in different genotype backgrounds***. **A**. Expression of the thyroglobulin (*TG*) gene. **B**. Expression of the thyroid-stimulating hormone receptor (*TSHR*) gene. For each thyroid gene, the entire cohort was divided into four genotype groups: no mutation, *BRAF* V600E alone, *TERT* promoter mutation alone, and the genetic duet of the two coexisting mutations. The central horizontal lines represent medians and the box boundaries represent interquartiles. The sample sizes in the no mutation, *BRAF* V600E, *TERT* promoter mutation, and the genetic duet groups were 149, 198, 11, and 28, respectively. *TERT* promoter mutations here included collectively *TERT* C228T and *TERT* C250T. *P* values were calculated using the two-sided Wilcoxon-Mann-Whitney test.

TABLE 1. Relationship between *BRAF* V600E or *TERT* promoter mutations and loss of radioactive iodine avidity in recurrent papillary thyroid cancer (PTC)

Tumor Type and	Loss of radioiodine avidity		Odds ratios (95% CI)	
Mutations Status	n/N (%)	P	Unadjusted	Adjusted*
All PTC				
BRAF V600E				
Negative	21/62 (33.9)		1.00	1.00
Positive	82/102 (80.4)	<0.001	7.88 (3.69-17.51)	7.11 (3.24-16.27)
TERT mutation				
Negative	61/117 (52.1)		1.00	1.00
Positive	42/47 (89.4)	<0.001	7.63 (2.75-26.46)	6.89 (2.28-25.66)
CPTC				
BRAF V600E				
Negative	10/39 (25.6)		1.00	1.00
Positive	71/87 (81.6)	<0.001	12.52 (4.82-35.27)	10.78 (4.03-31.38)
TERT mutation				
Negative	47/91 (51.6)		1.00	1.00
Positive	34/35 (97.1)	<0.001	31.23 (4.81-1322.43)	31.37 4.39-1402.18)

TERT promoter mutations here included collectively *TERT* C228T and *TERT* C250T.

* Adjusted for patient age and sex. CPTC, conventional PTC.

TABLE 2. Relationship between *BRAF* V600E alone, *TERT* promoter mutation alone or their coexistence and loss of radioiodine avidity in recurrent papillary thyroid cancer (PTC)

Tumor Type and	Loss of radioiodine avidity		Odds ratios (95% CI)	
Mutation Status	n/N (%)	P	Unadjusted	Adjusted*
All PTC				
No mutation	16/53 (30.2)	Ref.	1.00	1.00
BRAF V600E alone	45/64 (70.3)	<0.001	5.39 (2.31-13.13)	4.92 (2.07-12.20)
TERT mutation alone	5/9 (55.6)	0.251	2.84 (0.53-16.32)	2.10 (0.28-16.51)
BRAF + *TERT* mutations	37/38 (97.4)	<0.001	81.04 (11.67-3559.83)	103.68 (10.77-5771.67)
CPTC				
No mutation	8/36 (22.2)	Ref.	1.00	1.00
BRAF V600E alone	39/55 (70.9)	<0.001	8.30 (2.93-25.95)	7.33 (2.54-23.34)
TERT mutation alone	2/3 (66.7)	0.156	6.57 (0.31-427.22)	20.44 (0.22-2768.33)
BRAF + *TERT* mutations	32/32 (100.00)	<0.001	136.12 (20.82-+Inf)	179.58 (20.97-+Inf)

TERT promoter mutations here included collectively *TERT* C228T and *TERT* C250T.
*Adjusted for patient age and sex. CPTC, conventional PTC.

TABLE 3. Relationship between *BRAF* V600E alone, *TERT* C228T alone or their coexistence and loss of radioactive iodine avidity in recurrent papillary thyroid cancer (PTC)

Tumor Type and	Loss of radioiodine avidity		Odds ratios (95% CI)	
Mutation Status	n/N (%)	P	Unadjusted	Adjusted*
All PTC	103/164 (62.8)			
No mutation	16/53 (30.2)	Ref.	1.00	1.00
BRAF V600E alone	49/68 (72.1)	<0.001	5.96 (2.71-13.14)	5.63 (2.49-12.72)
TERT C228T alone	5/9 (55.6)	0.137	2.89 (0.69-12.20)	2.18 (0.39-12.25)
BRAF + *TERT* mutations	33/34 (97.1)	<0.001	76.31 (9.59-607.18)	127.69 (11.45-1423.89)
CPTC	81/126 (64.3)			
No mutation	8/36 (22.2)	Ref.	1.00	1.00
BRAF V600E alone	43/59 (72.9)	<0.001	9.41 (3.55-24.89)	8.83 (3.23-24.09)
TERT C228T alone	2/3 (66.7)	0.090	7.00 (0.56-87.50)	28.89 (0.77-1079.55)
BRAF + *TERT* mutations	28/28 (100.00)	<0.001	118.95 (18.10-+Inf)	162.36 (18.63-+Inf)

*Adjusted for patient age and sex. CPTC, conventional, PTC.

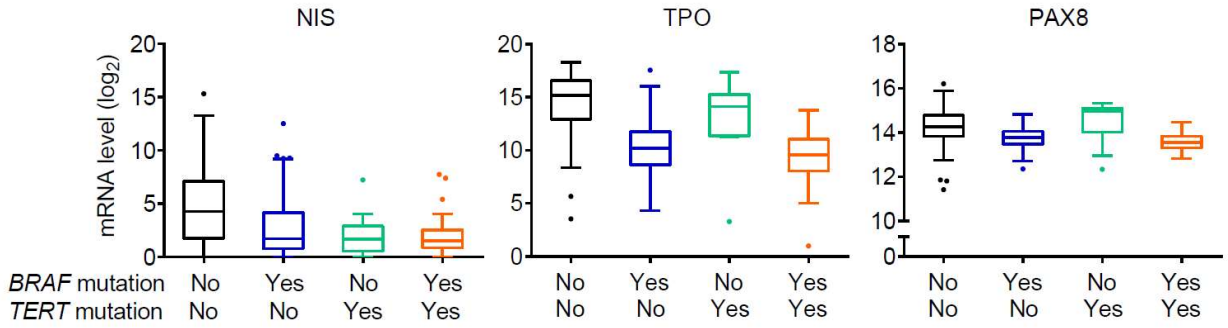
TABLE 4. Synergy test of interactions between *BRAF* V600E and *TERT* promoter mutations in their effect on the loss of radioactive iodine avidity in recurrent papillary thyroid cancer (PTC)

TERT Mutation	PTC Type	Risk of Loss of Radioiodine Avidity	
		Synergy Index (95% CI)	P
C228T and C250T	All PTC	13.28 (1.54-114.46)	0.019
	CPTC	--	--
C228T only	All PTC	10.99 (1.28-94.06)	0.029
	CPTC	--	--

The synergy index for the risk of loss of radioiodine avidity could not be calculated in the group of conventional PTC (CPTC) because loss of radioiodine avidity occurred in all of the cases harboring the genetic duet of coexisting *BRAF* and *TERT* mutations.

Supplemental Data

Liu J et al. The Genetic Duet of *BRAF* V600E and *TERT* Promoter Mutations Robustly Predicts the Loss of Radioiodine Avidity in Recurrent Papillary Thyroid Cancer.



Supplemental Figure 1. ***Box plots of mRNA expression of thyroid iodide-handling genes NIS, TPO and PAX8 in papillary thyroid cancer in different genotype backgrounds***. For each thyroid gene, the entire cohort was divided into four genotype groups: no mutation, *BRAF* V600E alone, *TERT* mutation alone, and the genetic duet of the coexisting two mutations. The central horizontal lines represent medians and the box boundaries represent interquartiles. The sample sizes in the no mutation, *BRAF* V600E alone, *TERT* promoter mutation alone, and the genetic duet groups were 149, 198, 11, and 28, respectively. *TERT* promoter mutations here included collectively *TERT* C228T and *TERT* C250T.